Halloysite Clay Nanotubes: Characterization, Biocompatibility and Use as Drug Carriers

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ABSTRACT

Halloysite is aluminosilicate clay with hollow tubular structure. Biocompatibility study is important for its potential application in controlled drug delivery. Halloysite nanotubes were added to different cell cultures for toxicity tests. Its fluorescence functionalisation by APTES and with fluorescently-labeled polyelectrolyte layers allowed following halloysite uptake by the cells with Confocal Laser Scanning Microscopy (CLSM). Quantitative Trypan blue and MTT measurements performed with two neoplastic cell lines model systems as function of the nanotubes concentration and incubation time indicate that halloysite exhibits a high level of biocompatibility and very low cytotoxicity, rendering it a good candidate for household materials and medicine. A combination of Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and Scanning Force microscopy (SFM) imaging techniques have been employed to elucidate the structure of halloysite nanotubes.

Keywords: halloysite, clay nanotubes, biocompatibility, TEM ,SEM

Introduction

There is an increasing amount of research on-going to produce functional nanometer-scale containers, and growing demand for their use in biomedical applications. Such containers would be inexpensive materials with a simple means of fabrication, thus calling for natural resources and nanotubes are good candidates for this. Halloysite clay is a two-layered aluminosilicate, chemically similar to kaolin, which has hollow tubular structure in the submicron range [1-3]. As for most natural materials, the size of halloysite particles varies of 50-70 nm in external diameter, ca 15 nm diameter lumen and 1 ± 0.5 μm length. Halloysite nanotubes are capable of entrapping a range of active agents within the inner lumen, followed by their retention and slow release [3-11].

Different chemistry of the inner and outer surfaces in halloysite tubes would also allow for separate modification of inner and outer walls, e.g., for selective labelling. The lumen diameter of halloysite tube fits well to macromolecule and protein diameters, allowing their encasing in the tube.

Biocompatibility is one of the main prerequisites for safe usage of halloysite in delivery of biologically active substances, in medical and household products. However, a comprehensive study of halloysite biocompatibility has not been done yet.

In this work, we focused on studying halloysite nanotubes interaction (both untreated and fluorescently labelled) with cells. We analysed halloysite toxicity and visualized the process of cell uptake of fluorescently labelled clay nanotubes.

Results

Halloysite Nanotubes Characterization

Halloysite nanotubes form stable colloids in water in wide pH range. Halloysite is negatively charged above pH 2.4 with zeta-potential reaching -50 mV at pH 6 and higher. High zeta-potential is a condition of good colloidal stability of dispersed nanotubes. An interesting feature is that due to different outside and inside chemistry (which comprise to silica, SiO_2, and alumina, Al_2O_3, sheets, correspondingly); the tube lumen is charged positively while external surface is charged negatively. This property of the tubes allows loading of negative molecules selectively into the lumen at the range of pH from 2.5 to 8.5. Scanning Electron Microscopy and Trasmission Electron Microscopy reveal that majority of the sample consists of cylindrical tubes of 40-50 nm diameter and length of 0.5 to 2 μm. HNTs are rather polydispersed in length. TEM images clearly indicate the empty lumen of the halloysite with 15 – 20 nm diameters. Halloysite nanotubes (HNTs) elasticity is considerably smaller than carbon nanotubes (CNTs) which have usually a Young's modulus of around 1 TPa [12-13]. Such large differences in elastic properties between CNTs and HNTs could facilitate HNTs internalization into cellular compartments, enhancing spontaneous penetration.

Imaging Halloysite Nanotubes Uptaken by the Cells

To study halloysite cytotoxicity we added these clay nanotubes (both fluorescently labelled and unlabelled) to two model cell cultures (MCF-7, breast cancer and HeLa, cervice cancer cells). Fig. 1 demonstrates halloysite uptake by cells.

Halloysite Cytotoxicity

Uncoated Halloysite: Fig. 2 summarizes data on cytotoxicity of halloysite studied with human cell models: breast cancer cell line (MCF-7) and cervice cancer cell (HeLa). We performed MTT tests at different time intervals (24h - 48h - 72h) and at different concentrations (from 1μg/mL up to 1 mg/mL). In both cell lines, the HNTs exhibited growth inhibition in a concentration-dependent manner. The cell viability was preserved (ca 70 % of cells survived) up to halloysite concentration of 100 μg/mL (Fig. 6-7). With increasing tube concentration up to 1000 μg\mL, there is a clear decrease of cell vitality (less pronounced for longer incubation time).

FITC Labelled Halloysite: To follow halloysite localization into the cells and to study its uptake we used nanotubes functionalized with FITC. Hela and MCF-7 cells mortality increases with increasing concentration for both cells in a similar manner with uncoated nanotubes. Therefore, uncoated and APTES functionalised halloysite nanotubes did not affect cell viability and the trends in the cell viability were the same. These toxicity data were also confirmed by Trypan blue tests performed at the same conditions. These data confimed the high biocompatibility of halloysite.

Discussion and Conclusion

With TEM, SEM and SFM microscopy, we characterized halloysite clay as tubular nanoparticles of ca 50 nm external diameter, 15 nm lumen diameter and of 500-1500 nm length. In wide range of pH it has negative electrical zeta-potential of ca -50 mV which allows halloysite good dispersibility and colloidal stability in water. An addition of halloysite to three different cell lines demonstrated that it is nontoxic up to concentrations of 100 ug/mL, while parallel laser confocal visualization of cell uptake of fluorescently labelled halloysite demonstrated its location within the cells in the nuclear vicinity. The current research suggests that HNTs is not toxic for cells.

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Figures:

Fig. 1 CLSM images of HNTs uptake by cancer cells

Fig. 2 MTT Test: Cytoviability of MCF7 (A) and HeLa (B) after interaction with HNT.

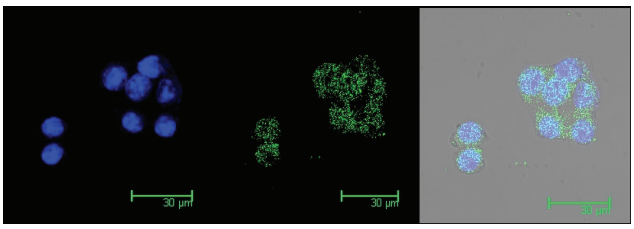

Fig.1

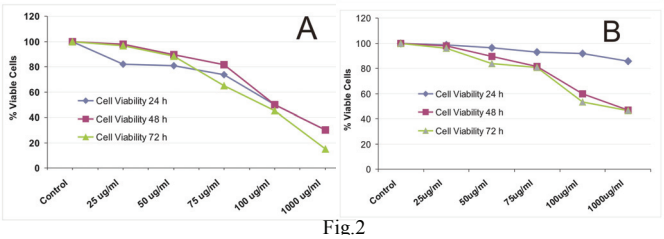

Fig.2